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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 10/069652
INTERNATIONAL APPLICATION NO. PCT/JP00/06342	INTERNATIONAL FILING DATE 18 Sept. 2000 (18.09.2000)	PRIORITY DATE CLAIMED 17 Sept. 1999 (17.09.1999)	
TITLE OF INVENTION FLUORESCENT PARTICLE IMAGING DEVICE			
APPLICANT(S) FOR DO/EO/US Yabusaki KATSUMI et al.			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information			
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371 3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1) 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau) b. <input type="checkbox"/> has been transmitted by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US) 6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)) 7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)) 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). 			
Items 11. to 16. below concern document(s) or information included:			
<ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included 13. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 14. <input type="checkbox"/> A substitute specification. 15. <input type="checkbox"/> A change of power of attorney and/or address letter 16. <input checked="" type="checkbox"/> Other items or information Form PCT/RO/101 Form PCT/ISA/210 Patent Cover Page 			

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DESCRIPTION

5 FLUORESCENT PARTICLE IMAGING DEVICE

Technical Field

The present invention relates to a fluorescent particle
imaging device, and more particularly to a fluorescent
10 particle imaging device that images fluorescent particles
such as white blood cells dyed with a fluorescent dye.

Background Art

In the field of medical treatment, since before, blood
15 platelet derivatives and red blood cell derivatives are being
manufactured by extracting blood platelets and red blood
cells from blood. These blood platelet derivatives and red
blood cell derivatives are each used for blood transfusions,
and it is not desirable to have white blood cells mixed therein.
20 For this reason, it is important to know the number of white
blood cells that are mixed in with such derivatives.
Conventionally, a sample of blood platelet derivative dyed
with a fluorescent dye is placed on a slide glass plate
referred to as a Nageotte chamber and irradiated with
25 illuminating light and the number of white blood cells is
counted using a microscope. Specifically, the number of white
blood cells in a 50 microliter sample is counted and converted
to the number of white blood cells in the whole bag. This
is a time-consuming task that has to be performed by an
30 experienced person, and is extremely inefficient and tiring.

JP 11-183382 A1 discloses a fluorescent particle imaging
device as a device that performs these tasks efficiently.
With this device, fluorescent particles (white blood cells)
dyed with a fluorescent dye are contained collectively at
35 the bottom of an imaging container, and only a portion near

5 The means disclosed here are very useful when used for
items such as blood platelet derivatives, blood plasma
derivatives, spinal fluid that have a high transmittance to
exciting light. Also, in the case of red blood cell
derivatives, centrifugal force is used to separate red blood
10 cells and white blood cells, and the removal rate is low,
at around 10%, leaving a large quantity of white blood cells
mixed in. In this case, the red blood cell derivative is
diluted and the reduced number of white blood cells in a
micro-sample is counted and, taking the dilution ratio into
15 consideration, converted to a whole-bag white cell count.
In this case the disclosed invention is useful because, even
though it is a red blood cell derivative, there is a high
transmittance to exciting light.

25 Therefore, an object of the present invention is to provide a fluorescent particle imaging device that can correctly count the number of fluorescent particles even when the measurement target substance has a different transmittance to exciting light.

Disclosure of Invention

In accordance with the present invention, to attain the above object, an imaging device for imaging fluorescent particles dyed with a fluorescent dye comprises an imaging
35 container for containing fluorescent particles collectively

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at the bottom, means for producing exciting light that excites the fluorescent particles, first illumination means for irradiating only a portion near the bottom of the imaging container with the exciting light from a side, second
5 illumination means for irradiating the bottom of the imaging container from the bottom with the exciting light, switching means for switching the first and second illumination means, and an imaging device for imaging, from the bottom, the bottom of the imaging container illuminated by the first or second
10 illumination means.

If the container contains a substance having a high transmittance to exciting light such as a blood platelet derivative or a diluted red blood cell derivative and a portion is irradiated other than where the fluorescent
15 particles are collected, the presence of fluorescent dye there too will result in background light that degrades the contrast of the obtained image. In such a case, it is therefore preferable to irradiate only the portion near the bottom of the container from the side.

20 On the other hand, if a substance having a low transmittance to exciting light such as a non-diluted, high-concentration red blood cell derivative is irradiated from the side, fluorescence will be obtained on the side of exciting light incidence, but the exciting light hardly
25 reaches the opposite side, making it difficult to obtain sufficient fluorescence. It is therefore preferable to irradiate the bottom of the container from the bottom. In this case, the exciting light reaches only the portion near the bottom because of low transmittance, and hardly any
30 fluorescence acting as background light is produced.

With the present invention, the illumination means are switched so that for a substance having a high transmittance to exciting light, only the portion near the bottom of the container are irradiated from the side with the exciting light,
35 and for a substance having a low transmittance to exciting

Brief Description of Drawings

Figure 2 is a frontal view showing a device for analyzing and displaying images of picked up fluorescent particles.

15 The invention will be explained in detail below with
reference to the embodiment as shown in Figures.

Figures 1 and 2 show an embodiment of the present invention. In the drawings, reference numeral 1 denotes a laser light source such as a YAG laser that produces a green-wavelength laser beam. A switchable mirror 20 removable from the optical path by a switching mechanism (not shown) is disposed on the optical path of the laser beam from the laser light source 1. When the switchable mirror 20 is removed from the optical path, the laser beam from the laser light source 1 falls incident on, and is diffused by, a member having a diffusing function such as a diffuser 2 constituted by ground glass or the like, and irradiates, from the side, the bottom 3' of an imaging container 3, the top of which is covered by a cover 4. Fluorescent particles are collected at the bottom of the imaging container 3. The irradiation of the fluorescent particles with the laser beam causes fluorescence to occur. An image of the fluorescent particles irradiated by the laser beam passes through an objective lens 6, is reflected by a mirror 7, and after passing through a

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barrier filter 8 that transmits light of a prescribed frequency band, is picked up by a CCD camera 9.

As shown, when the switchable mirror 20 is placed in the optical path, the exciting light from the laser light source 1 is reflected by the switchable mirror 20 and a mirror 21 and then diffused by a diffuser 22 similar to the diffuser 2 to irradiate the bottom of the imaging container 3 from below. The images of the fluorescent particles irradiated by the laser beam pass through the objective lens 6, are reflected by the mirror 7, and after passing through the barrier filter 8 that transmits light of the prescribed frequency band, are picked up by the CCD camera 9.

As shown in Figure 2, the images of the fluorescent particles picked up by the CCD camera 9 are input via a signal line 10 to a computer 12 by means of the computer's video capture facility 11. In the computer 12, the images are processed by an image processing circuit 13, whereby the fluorescent particles are recognized. This recognition is made possible by the fact that the presence of a fluorescent particle causes a change in brightness and the differentiation of signal values, for example, allows the coordinate position of fluorescent particles to be detected. The fluorescent particles thus recognized are displayed on a monitor 14. Figure 2 shows that an image 15 of the bottom of the container and a plurality of fluorescent particles 15a are displayed on the monitor 14. The number of fluorescent particles 15a is counted and the count value is also shown at the lower part 16 of the monitor 14.

The imaging container 3 is integrally formed of transparent polystyrene resin, glass or acrylic resin, but preferably polystyrene resin. Blood platelet derivative, for example, or diluted or non-diluted red blood cell derivative or the like can be placed in the imaging container 3. A chemical solution (Triton X (trademark)) for dissolving the cytoplasm of platelets and white blood cells, and a

In such an arrangement, when a substance having a high transmittance to exciting light, such as a blood platelet derivative or a times-hundred dilution of a red blood cell derivative, is placed in the imaging container 3, the switchable mirror 20 is removed from the optical path and the laser light source 1 is switched on to irradiate only the portion near the bottom of the imaging container 3 with exciting light from the side. The white blood cells dyed with a fluorescent dye are collected at the bottom of the imaging container 3. The dyed nuclei of the white blood cells are therefore excited by the incident laser beam, producing the fluorescence at around 600 nm. The fluorescent light passes from the bottom of the container through the objective lens 6, the mirror 7 and the barrier filter 8 and is picked up by the CCD camera 9. The barrier filter 8 only transmits light in the fluorescence wavelength band, making it possible to block disturbing-wavelength light.

25 The laser beam only irradiates the bottom of the container where the fluorescent particles are collected, and effectively irradiates the white blood cells collected at the container bottom from the side. Therefore, even if fluorescent dye is suspended in the solution in the container
30 3, the fluorescence is prevented from acting as a disturbing background light, improving the contrast of the images that are picked up. As shown in Figure 2, the images of the fluorescent particles picked up by the CCD camera 9 are input via the signal line 10 to the computer 12 by means of the
35 computer's video capture facility 11 and processed by an image

When, on the other hand, the imaging container 3 contains a non-diluted, high-concentration red blood cell derivative, the switchable mirror 20 is inserted into the optical path so that the exciting light from the laser light source 1 may be reflected upwards by the mirror 21 to irradiate the bottom of the imaging container 3 from below with the exciting light. As a result, the white blood cells collected at the bottom of the container emit fluorescent light and, as in the case of irradiation from the side, the number of white blood cells is counted by counting the number of fluorescent particles. The exciting light is absorbed by a red blood cell derivative, which exhibits low transmittance thereof. Therefore, the exciting light reaches only the portion near the bottom and hardly any fluorescence acting as background light is produced even when it is irradiated from the bottom. This enables high-contrast images to be obtained and also enables the number of white blood cells to be counted correctly.

25 The above embodiment has been described with reference
to a beam from a single laser light source that is switched
by a switchable mirror to irradiate the bottom of the
container from the side or from the bottom. Instead of this,
separate laser light sources can be provided to effect
30 irradiation from the side and irradiation from the bottom.
In this case, when the bottom of the container is to be
irradiated from the side the laser light source for that
purpose would be switched on, and when the container is to
be irradiated from the bottom the laser light source for that
35 purpose would be switched on. Alternatively, both laser light

CLAIMS

1. An imaging device for imaging fluorescent particles dyed with a fluorescent dye, comprising:

5 an imaging container for containing the fluorescent
particles collectively at the bottom;

means for producing exciting light that excites the fluorescent particles;

first illumination means for irradiating only a portion
10 near the bottom of the imaging container with the exciting
light from a side;

second illumination means for irradiating the bottom of the imaging container from the bottom with the exciting light;

15 switching means for switching the first and second
illumination means; and

an imaging device for imaging, from the bottom, the bottom of the imaging container illuminated by the first or second illumination means.

20

2. The fluorescent particle imaging device according to claim 1, wherein the switching means is an optical element that in a first switch position guides the exciting light to a side of the imaging container bottom, and in a second switch position guides the exciting light to the bottom of the imaging container.

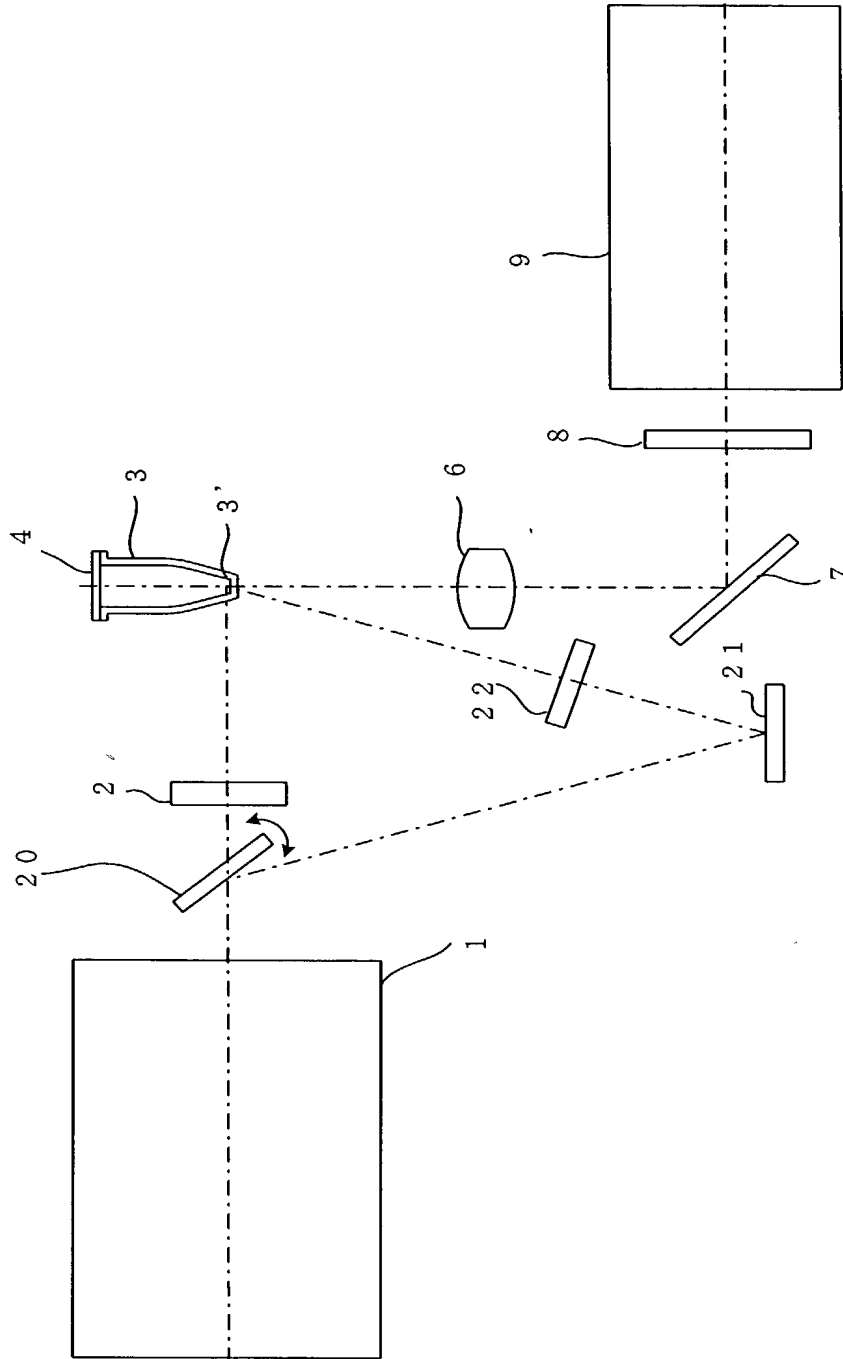
3. The fluorescent particle imaging device according to claim 1, wherein an exciting light source for side irradiation of the imaging container bottom and an exciting light source for bottom irradiation of the imaging container are provided, and the switching means is used to switch to the exciting light from one of the exciting light sources.

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ABSTRACT

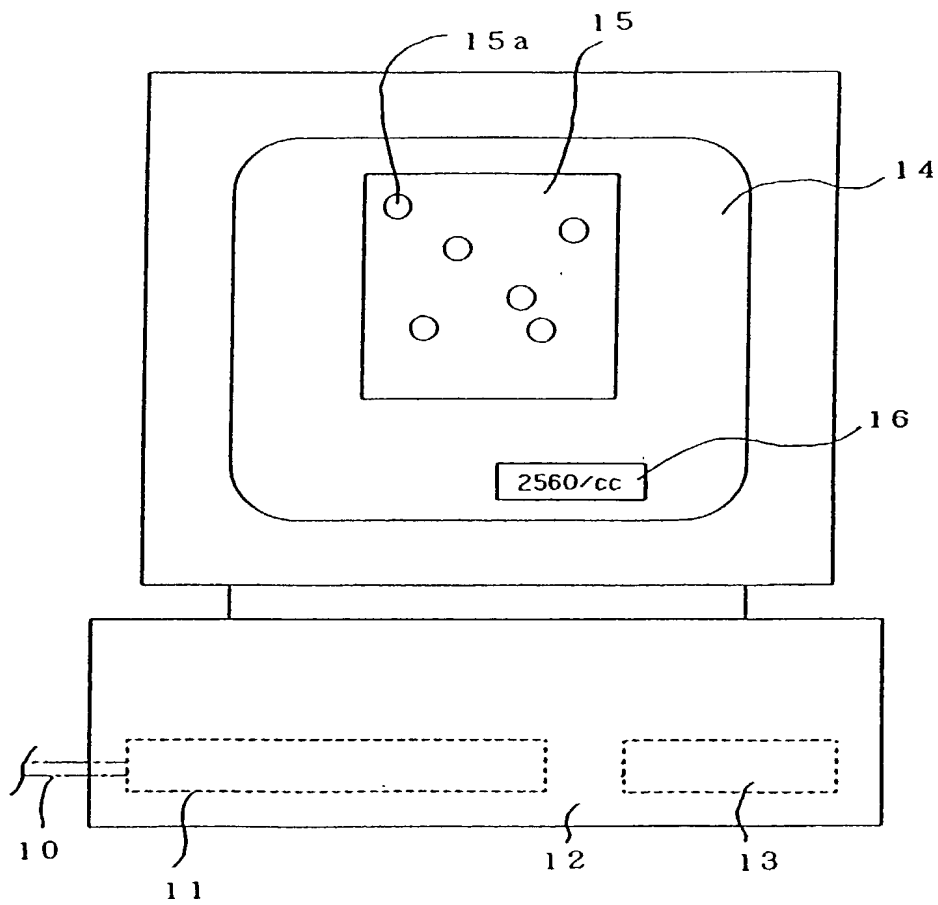
An imaging container (3) for containing fluorescent particles dyed with a fluorescent dye collectively at the bottom is provided. If the container contains a substance having a high transmittance to exciting light such as a blood platelet derivative or a diluted red blood cell derivative, a mirror (20) is removed from the optical path, and only the portion near the bottom of the container are irradiated from a side with the exciting light. Even if the solution in the container contains a fluorescent dye, the fluorescence is prevented from acting as disturbing light. If the container contains a substance having a low transmittance to exciting light such as a high-concentration red blood cell derivative, the mirror (20) is placed in the optical path, and the bottom of the container is irradiated from below. In this case, since transmittance is low, the exciting light reaches only the portion near the bottom, and fluorescence acting as background light is hardly produced. Even if the transmittance to the exciting light is different, illumination corresponding to the transmittance can be selected, and therefore the fluorescent particles can be counted correctly.

FIG. 1



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FIG. 2



DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

FLUORESCENT PARTICLE IMAGING DEVICE as described and claimed in

PCT/JP00/06342 filed September 18, 2000

the specification of which (check one); ☒ is attached hereto; ☐ was filed on _____ as Application Serial No. _____ and was amended on (or amended through) _____ (if applicable). I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
✓ 11-263145	Japan	17/09/1999	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
____	____	____	<input type="checkbox"/> Yes <input type="checkbox"/> No
____	____	____	<input type="checkbox"/> Yes <input type="checkbox"/> No
____	____	____	<input type="checkbox"/> Yes <input type="checkbox"/> No
____	____	____	<input type="checkbox"/> Yes <input type="checkbox"/> No
____	____	____	<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status - Patented, Pending or Abandoned)
____	____	____
(Application Serial No.)	(Filing Date)	(Status - Patented, Pending or Abandoned)
____	____	____

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY

I (we) hereby appoint Bruce L. Adams, Registration No. 25,386, Van C. Wilks, Registration No. 25,027 and Franco S. De Liguori, Registration No. 36,497, whose post office address is: Adams & Wilks, 50 Broadway, 31st Floor, New York, New York 10004, as my (our) attorneys with full power of substitution and revocation, to prosecute this application, and to transact all business in the United States Patent and Trademark Office connected therewith.

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